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Working Group 4. Drug- and Toxicant-Induced Liver Disease

Introduction & Background

Drug- and toxicant-induced liver disease has emerged in recent years as an increasingly important health problem in the U.S. Hepatotoxicity from foreign compounds (xenobiotics), such as drugs, herbal products, or environmental chemicals, now represents the leading cause of acute liver failure, and is the most common reason for withdrawal of an approved medication from clinical use. The increasing incidence of hepatotoxicity appears to be due to the greater use of prescription and over-the-counter medications and herbal remedies.

The clinical presentation of drug hepatotoxicity mimics all forms of acute and chronic liver disease. Drug hepatotoxicity that simulates acute hepatitis can be life-threatening or can induce chronic liver disease, whereas those drugs that cause a cholestatic form of liver disease rarely result in death, but may lead to a protracted course, culminating in the vanishing bile duct syndrome or even biliary cirrhosis. Drugs can also cause acute fatty liver, granulomatous hepatitis, and chronic hepatitis that progresses to cirrhosis and liver cancer. The diversity of clinical presentations of drug-induced liver disease reflects the variety of drugs that can harm the liver and their multiple pathways of injury. The clinical and pathologic presentation and latency associated with an individual drug is referred to as its "signature." Although signatures are often useful in providing support for attribution of causality, variability in clinical manifestations is also common, limiting the utility of this approach.

The pathophysiology of hepatotoxicity has been elucidated for only a few drugs, at least in part, and several chemical substructures have been identified as pro-toxicants in that they can be metabolically transformed into toxic reactive metabolites. Nevertheless, the molecular mechanisms of injury for most drugs are still poorly understood. Consequently, it is difficult to predict or prevent liver injury due to drugs. Furthermore, for most forms of drug-induced liver disease, the only "therapy" is early identification and immediate removal of the drug. There are few specific antidotes for drug hepatotoxicity, with the exception of N-acetylcysteine for acetaminophen overdose and possibly L-carnitine for valproic acid hepatotoxicity.

Drug-induced liver injury can be separated into two forms—predictable and unpredictable. With the exception of acetaminophen-associated liver injury, most hepatotoxicity seen today is unpredictable and idiosyncratic, occurring only rarely in individuals who receive the medication. Because the liver injury depends upon human idiosyncratic differences, it is rarely predicted by preclinical animal toxicology and may only become evident once the medication is introduced widely into practice. Idiosyncratic drug reactions occur in two general patterns: (1) allergic, in which liver injury is accompanied by features of hypersensitivity (e.g., rash, eosinophilia, latency of less than 2 months, anamnestic response to rechallenge, HLA associations, and presence of autoantibodies); or (2) non-allergic, in which allergic features are absent, metabolic abnormalities may play a role, and the latency is variable, but often prolonged (e.g., 2–12 months). In both types of idiosyncratic reactions, overt liver disease may occur on a background of more frequent, asymptomatic and mild injury, which often resolves with continued use of the

drug. These features suggest that severe liver injury arises when there is a lack of adaptation to the mild injury caused by the drug or when there is a second, possibly unrelated factor that accelerates the acute injury. Periodic monitoring of serum aminotransferase activities during therapy with potentially hepatotoxic drugs has been recommended by the Food and Drug Administration to identify early evidence of liver injury in this situation, with the hope of avoiding more severe outcomes. The efficacy of this approach is unproven, but is likely to be compromised by the potential for rapid progression of injury; by poor compliance by physicians with monitoring recommendations; and by the fact that it is difficult to distinguish between elevated serum enzyme levels that reflect true hepatotoxicity and those that occur transiently as a result of an adaptive response to the drug.

A significant problem in this field of hepatotoxicity is proof of causality. Aside from placebo-controlled clinical trial databases, which rarely have enough cases to identify rare adverse events, it is difficult to prove cause-and-effect relationships between use of a drug and liver injury. Most information on hepatotoxicity in humans is based upon individual case reports and small case series in which the denominator is unknown and the background incidence of idiopathic liver injury in the specific disease population is poorly defined. Several causality assessment scales have been developed that weigh such factors as latency, rate of resolution after discontinuation, exclusion of other diseases, risk factors, current knowledge of the potential for hepatotoxicity, rechallenge, and immunoallergic features. These approaches could benefit from optimization and validation in a prospective fashion.

Recent Research Advances

Over the past few decades, considerable progress has been made in understanding the pathogenesis of drug-induced liver disease. In early studies, the hepatotoxicity due to acetaminophen was attributed to its activation in the liver to reactive metabolites that caused depletion of glutathione (GSH), with subsequent reaction of the unconjugated metabolites to critical cellular macromolecules. Subsequently, progress has been made in characterizing reactive metabolite pathways for other drug structures and chemical substructures. Drug uptake, metabolism, and secretion is a multi-step process. The initial step is usually accomplished by the cytochrome P450 (CYP) or “phase 1” enzymes, which are critical in normal drug metabolism. The “phase 2” conjugating enzymes include GSH S-transferases, sulfotransferases and glucuronosyl transferases. These enzymes are responsible for conjugation of the reactive intermediates produced by the phase 1 enzymes, aiding in their detoxification and excretion. More recently, there have been major developments in defining membrane transporters (“phase 3” proteins), which control the cellular entry and exit of drugs and their metabolites. The role of transcription factors, which regulate the constituents, has also been partially elucidated, including orphan nuclear receptors, such as SXR and CAR. Thus far, however, only limited information is available on the polymorphisms of phase 1, 2 and 3 constituents and their roles in determining or predicting risk of hepatotoxicity.

Progress in other areas of investigation has begun to shed light on hepatotoxicity. In particular, progress has been made in understanding the mechanisms of cell apoptosis, oxidative stress, mitochondrial function, cytokine pathways and actions, the roles of the liver nonparenchymal

cells (i.e., stellate, endothelial and Kupffer cells) and the innate immune system. Thus, hepatotoxicity may not be a simple result of initial modification of intracellular macromolecules, organelles or membranes, but may occur as a result of the triggering of cell death signaling pathways. These advances have provided targets for further investigation of hepatotoxicity. Use of experimental models has been helpful in defining the effects of genetics and environment, including concomitant diseases, on oxidative stress responses, cell death pathways, and the status of innate immune responses.

Clinical research on hepatotoxicity has defined a variety of genetic and environmental risk factors for drug-induced hepatotoxicity. These risk factors tend to be drug-specific, and include age, gender, obesity, and underlying diseases, such as HIV, viral hepatitis, and alcoholism, as well as *CYP* polymorphisms, cytokine expression levels, and HLA subtypes. Perhaps as important, hepatotoxicity research has been helped by improved diagnostics for other forms of liver disease, such as viral hepatitis. The ability to exclude other diagnoses allows for a clearer understanding of what qualifies as a drug-induced liver disease.

Research Goals

The major goals of research on drug- and toxicant-induced liver disease are to establish means of predicting the likelihood of hepatotoxicity due to drugs, herbal medications, and environmental toxicants; to improve diagnostic ability; to elucidate the mechanisms of hepatotoxicity; and to develop means of preventing and treating liver cell injury.

Pathogenesis and Animal Models: The greatest challenge in this area is to elucidate the causes and mechanisms of injury in idiosyncratic forms of drug- and toxicant-induced liver disease. Examples of drugs that cause such injury include isoniazid, rifampin, clavulanic acid, erythromycin, sulfonamides, phenytoin, carbamazepine, valproic acid, amiodarone, and several of the commonly used nonsteroidal anti-inflammatory agents and statins. The development of animal models or *in vitro* systems for the study of the toxicities of these drugs would facilitate this research (Matrix Cell A3). An important goal is to develop an animal model of adaptation to drug-induced liver injury that shows early, mild injury, such as minor serum aminotransferase elevations, that resolves even with continuation of the medication (Matrix Cell B3). Failing to achieve such adaptation or sustaining an additional injury due to a second triggering mechanism may well explain why the rare patient develops overt hepatotoxicity while many others have subclinical liver injury that resolves. Animal models of allergic forms of hepatotoxicity would also be valuable. Molecular methods for manipulating immune regulation and hypo- or hyper-activity of the innate or acquired immune system may provide a mechanism for generating these models (Matrix Cells A3 and B3). Of particular value would be the development of “human susceptible” mouse models to investigate compounds that are hepatotoxic in specific human populations but not in normal animal models. Creation of such complex animal model systems is difficult and high-risk, and, therefore, would be helped by resource-developing efforts.

Hepatotoxicity due to drugs and herbal medications is likely to be mediated through the normal cell processes that cope with stress and injury. Indeed, hepatotoxicants are often employed to investigate normal cell biology. In like manner, studies of normal cell biology may be useful in

investigating the causes of both predictable and unpredictable hepatotoxicity. The pathways for predictable injury are particularly approachable using agents, such as acetaminophen, nucleoside analogues, and alcohol, which can cause acute hepatocellular necrosis, mitochondrial damage, and oxidative stress. More work is warranted to identify critical protein targets of reactive metabolites of these agents and to define their functional significance. The cell signaling pathways involved in programmed cell death, oxidative stress, molecular transport and trafficking, and organelle and mitochondrial function provide potential targets to investigate the underlying mechanisms of hepatotoxicity and to develop possible means of prevention or treatment of drug-induced liver disease (Matrix Cell B2). An important use of animal model systems is the ability to identify chemical substructures that are protoxicant, which can then be avoided in new drug development (Matrix Cell A3).

Clinical Investigation: Most hepatotoxicity is unpredictable and idiosyncratic. Therefore, further progress in this area will depend upon clinical investigation focusing upon patients with hepatotoxicity as well as asymptomatic controls to assess drug metabolism and patterns of injury using comparative studies of transcriptomics, proteomics, metabolomics, and genomics. The potential for success of such studies would depend upon the availability of serum, tissue, RNA, and DNA samples from well-characterized cases of hepatotoxicity caused by drugs and herbal medications (Matrix Cell B1). Most deserving of careful assessment are the host, environmental, immunologic, and genetic factors that play an important role in provoking or enhancing the liver injury. Genetic factors are probably of great importance in idiosyncratic forms of hepatotoxicity and are appropriately studied using advanced genomic and genetic methodology (Matrix Cell C1). Genetic linkage studies of family members who had similar reactions to medications would be valuable as well. Samples from established cases could also be used to define molecular signatures for liver injury caused by drugs (Matrix Cell C3).

A great challenge clinically in the management and study of hepatotoxicity is the difficulty in assigning causality, proving that the injury is due to the medication and not due to an unknown or unsuspected liver disease. Many cases of drug-induced liver injury occur in patients with complex medical histories who have several possible causes for liver disease. The current instruments for assessing causality are imperfect and not widely used. A focused effort on developing strict definitions and conveniently standardized means of evaluating hepatotoxicity would be of great value (Matrix Cell A1).

Better clinical definitions and standardization of terms used in reporting hepatotoxicity cases would also allow for a better understanding of the frequency and burden of this condition in the U.S. (Matrix Cell B2). Most population-based studies, even if they deal partially with the prevalence and incidence of liver disease, do not provide information on the incidence and severity of liver disease caused by drugs. In the area of acute liver failure, careful prospective data are being accrued on cases in the Acute Liver Failure Study Group, which has shown that drug-induced liver injury accounts for over half of acute liver failure in the U.S. Similar systems for assessing the proportion of drug-induced acute jaundice, chronic liver disease, cirrhosis and hepatocellular carcinoma would be helpful, and, in many situations, such systems could easily be added to ongoing epidemiological studies or surveys. In a similar fashion, the incidence of specific forms of hepatotoxicity could be a focus of any clinical trial of medication, and data could be acquired that allow for assessment of risk factors (e.g., age, sex, race and underlying

illness). Similarly, underlying liver disease (e.g., chronic hepatitis or nonalcoholic steatohepatitis) is often cited as a risk factor and a reason not to use a potentially hepatotoxic drug, even though the hepatotoxicity is idiosyncratic and its link to underlying disease unproven. Therefore, it is important that the relative risk of liver injury be studied, not only in persons who have no pre-existing liver disease, but also in those with chronic liver disease and that clinical trials of new medications not limit enrollment to patients with normal serum aminotransferase levels.

Diagnosis of drug-induced liver disease is currently based on exclusion of other causes of liver injury. The diagnosis of hepatotoxicity would be enhanced by the availability of a specific "signal" or "signature" of liver disease. Such a signal is important for: 1) predicting the likelihood of development of hepatotoxicity, and 2) confirming the early diagnosis of true hepatotoxicity when elevated enzyme levels are observed and distinguishing it from transient elevations associated with an adaptive hepatic response (Matrix Cell C2). While a single signature is unlikely to be identified for all forms of hepatotoxicity, characteristic signals or biomarkers may be found for specific forms (e.g., hepatitis-like, cholestatic, fatty-liver). The methods of proteomics, metabolomics and transcriptomics could be applied to cases of hepatotoxicity and appropriate disease controls to search for a positive diagnostic assay (Matrix Cells C3 and C2). Identification of metabolic products of medications associated with liver injury might provide positive diagnostic assays for hepatotoxicity associated with certain medications, such as acetaminophen (Matrix Cell A2).

Prevention and Therapy: Until more is known about the mechanisms and risk factors for hepatotoxicity, it is unlikely that means of prevention or therapy can be developed. For liver injury that appears to be due to drug-hypersensitivity, immunosuppressive agents are often used, and clinical trials to evaluate the efficacy of this approach are warranted. For other forms of hepatotoxicity, nonspecific hepatoprotective agents or approaches that improve pathways of cell stabilization or adaptation may be effective and deserve to be tested (Matrix Cell C2). Other examples of nonspecific agents that might ameliorate the course of hepatotoxicity include N-acetylcysteine, S-adenosyl methionine, antioxidants, promoters of liver regeneration, and inhibitors of proinflammatory cytokines. With better elucidation of the pathways involved in common forms of hepatotoxicity, more suitable means of treatment may become available. Until then, the best approach is prevention. Public health and education measures are appropriate, particularly for hepatotoxicity from acetaminophen, which often occurs as a result of unintentional overdose, with a patient unknowingly taking too much acetaminophen over the course of several days and presenting later with severe liver injury. Research on standardization of dose size, labeling, packaging and educational programs might be helpful in decreasing acetaminophen hepatotoxicity, which is the single major cause of acute liver failure in the U.S.

Steps to Achieve Research Goals

The area of drug-induced liver disease research would be greatly helped by increased collaborations between clinical and basic investigators. Efforts could be made to facilitate basic research investigations using material from clinical studies of drug-induced liver disease and to test new hypotheses in *in vitro* and *in vivo* systems, as well as in humans. Such cross-fertilization is essential for progress in hepatotoxicity research and is an important example of the benefits of both bench-to-bedside and bedside-to-bench (bi-directional translation) research.

Investigation of hepatotoxicity would benefit from the development of a database of well-characterized clinical cases of drug-induced liver disease matched with serum, urine, tissue, and RNA and DNA samples that are available for investigations using state-of-the-art methods of genomics, transcriptomics, proteomics, and metabolomics. A chemical structure and substructure database of drugs that cause liver injury would be supportive of this effort and might lead to the future development of safer drugs. A network for drug-induced liver disease has recently been developed, called the Drug-Induced Liver Injury Network (DILIN). This Network is developing means of accrual, evaluation and follow-up of community-acquired cases of hepatotoxicity that display either specific hepatotoxic symptoms or jaundice. A primary purpose of the Network is to develop interactions with the entire community of hepatotoxicology investigators to provide materials for human investigation. This Network could also be used to evaluate new hypotheses and new approaches to categorization, diagnosis, assignment of causality, management and treatment.

Hepatotoxicity is a major concern of pharmaceutical companies involved in new drug development. Drug-induced liver disease is the single most common reason for withdrawal of an approved medication from clinical use and is an important reason for failure of new drugs in preclinical and clinical evaluation. The pharmaceutical industry has enormous databases on chemical structures that can cause liver injury and other resources useful in researching hepatotoxicity. Efforts could be made to develop scientific collaborations on specific issues in hepatotoxicity, such as the development of animal models and prospective screening methods, as well as establishing the incidence of different forms and degrees of injury with specific medications. The increased use of post-marketing surveillance as a means of identifying uncommon adverse reactions to medications is an important advance and can be used more actively to elucidate the mechanisms of hepatotoxicity. These studies could also be used to evaluate the effectiveness of screening for aminotransferase elevations in patients placed on potentially hepatotoxic drugs, an approach that has been recommended for several medications, but is expensive, cumbersome and of unproven benefit. To facilitate this collaboration, regular scientific meetings that include investigators from industry, academia and government could be encouraged. New and innovative ways of supporting research on hepatotoxicity and training in this area are also important steps for ensuring progress in research on drug-induced liver disease.

Matrix of Research Goals in Drug- and Toxicant-Induced Liver Disease

	Short-term Goals (0-3 years)	Intermediate-term Goals (4-6 years)	Long-term Goals (7-10 years)
High Risk	**A3. Develop *in vitro* or *in vivo* systems for study of allergic & nonallergic idiosyncratic hepatotoxicity.** **Identify chemical sub-structures that are protoxicant & could be avoided in design of new drugs.**	**B3. Define role of innate immune system in both allergic and non-allergic forms of hepatotoxicity.** **Develop an animal model of adaptation to hepatotoxicity to help define the genes necessary for the adaptive response.**	**C3. Develop molecular signatures that are diagnostic for major forms of hepato-toxicity.**
Intermediate Risk	**A2. Develop positive diagnostic assay for acetaminophen toxicity.**	**B2. Elucidate molecular mechanisms of common forms of hepatotoxicity.** **Define incidence of drug-induced liver injury and the contribution of hepatotoxicity to the burden of acute & chronic liver disease in the U.S.**	**C2. Determine the efficacy of nonspecific therapy of hepato-toxicity with antioxidants or hepatoprotective medications.** **Develop and assess biomarkers or metabolites to predict the development of hepatotoxicity, and to distinguish between established hepatotoxicity and transient, adaptive enzyme elevations.**
Low Risk	**A1. Develop definitions & standardization of procedures for diagnosis of hepatotoxicity and assignment of causality.**	**B1. Develop a cohort of patients with well-characterized hepato-toxicity and controls with availability of serum, tissue, RNA & DNA for transcriptomic, proteomic, metabolomic & genomic studies.**	**C1. Identify genetic factors that contribute to hepatotoxicity of several major forms of drug-induced liver disease.**